APPENDIX A

Summary of references for role of RNA fragments in silencing (all organisms, not only plants)

Year	Citation		
1991	Fire et al., Development 113:503-14		disruption of expression of myofilament proteins C. elegans (nematode) by fragments of unc-22 and unc-54 in antisense and sense ortientation
1994	Cogoni et al., Antonie Van Leeuwenhoek 65:205-9		transformation of Neurospora (fungus) with fragments of carotenogenic albino 1 or albino 3 genes results in suppression
1996	Cogoni et al., EMBO J 15:3153-63		fragments with approximately 132 bp of sequences homologous to the transcribed region of the native gene are sufficient to produce sense suppression in Neurospora (fungus)
1997	Mtezlaff et al., Cell 88:845-54		sense suppression of chalcone synthase involves 43-base paired segments of the coding region and 3' UTR of the transgene transcript that are 80% complementary.
1997	Ratcliff et al., Science 276:1558–1560		virus cross-resistance and transgene-induced gene silencing in plants involve similar RNA-based mechanisms
1998	Fire et al., Nature 391:806-11		double-stranded RNA corresponding to fragments of exxons inhibits gene expression in C. elegans (nematode) more effectively than sense or antisense single-stranded RNA
1998	Kennerdell and Carthew, Cell 95:1017-26		double-stranded RNA corresponding to fragments of genes interferes with gene expression in Drosophila (fruit fly) embryos
1998	Montgomery and Fire, Trends Genet 14:255-8	Review	
1999	Cogoni and Macino, Curr Opin Microbiol 2:657-62	Review	commonalities of homology-dependent gene silencing in fungi and plants
1999	Hamilton and Baulcombe,Science 286:950-952		antisense and sense small RNAs ca. 25 nucleotides are present in tomatoes with cosuppression or antisense suppression
2001	Elbashir et al., Genes Dev 15:188-200		21- and 22-nt RNA fragments generated from double-stranded RNAs are the sequence-specific mediators of RNA interference in Drosophila (fruit fly)
2001	Thomas et al., Plant J 25:417-25		synthetic nucleotides with 23 - 30 base matches to gfp transgene were effective in VIGS; sequences as short as 33 nt silenced endogenous phytoene desaturase
2002	Han and Grierson Plant J 29:509-519		Small antisense RNAs, about 23 nucleotides, present in transgenic tomato plants exhibiting post-transcriptional silencing of the endogenous polygalacturonase
2004	Baulcombe, Nature 431:356-63	Review	cytoplasmic siRNA silencing (virus resistance), the silencing of endogenous messenger RNAs by miRNAs, and silencing via DNA methylation in plants all involve the cleavage of a doublestranded RNA (dsRNA) into short 21–26-nucleotide RNAs

APPENDIX A

Suppression of gene expression in plants by fragments of gene coding sequences

Year	Citation	Gene	Type	Length	Comments
1990	Smith et al., Mol Gen Genet 224:477-81	polygalacturonase	sense	truncated transcript	
1995	Kumagai et al., Proc Natl Acad Sci U S A 92:1679-83	phytoene desaturase	VIGS sense	651 bp	partial cDNA (Arg208-Thr424) from tomato, worked in N. benthamiana
			VIGS antisense	651 bp	partial cDNA (Arg208-Thr424) from tomato, worked in N. benthamiana
1996	Hamada et al., Transgenic Res. 5:115-21	omega-3 fatty acid desaturase	antisense	0.5 kb	3'-flanking region and a part of the coding region
1997	Song et al., Plant Physiology 114:927-935	Peptide Transport Gene AtPTR2-B	antisense		SacI-XbaI fragment of AtPTX2-B, has 100-bp deletion of the coding region and lacks 230 bp from 3' noncoding region of cDNA
			sense		
1997	Fan et al., Plant Cell 9:2183-2196	phospholipase D alpha	antisense	786	DNA fragment nucleotides 1446 to 2231 of cDNA
1998	Ruiz et al., Plant Cell 10:937–946	phytoene desaturase	VIGS	415 nt	
			VIGS	377 nt	distinct region from 415 nt fragment
			VIGS	212 nt	from within 415 nt fragment
1998	El Euch et al., Plant Mol Biol 38:467-79	chalcone synthase	antisense	400 bp	
1999	Brugiere et al., Plant Cell 11:1995-2012	cytosolic glutamine synthetase	antisense	274 bp	
1999	D'Aoust et al., Plant Cell 11:2407–2418	sucrose synthase	antisense	538 bp	
2001	Thomas et al., Plant J 25:417-25	gfp transgene	VIGS - antisense	23 - 30 nt	
			VIGS - sense	23 - 30 nt	
		phytoene desaturase	VIGS - antisense	33, 51, 52 nt	
			VIGS - sense	33, 51, 368 nt	
2002	Stoutjedijk et al., 2002 Plant Physiol	delta-12-desaturase	inverted repeat	120 bp	
			inverted repeat	480 bp	
			sense	1103 bp	
2003	Laurie et al., J Exp Bot 54:739-747	SNF1-related protein kinase-1	antisense	527 bp	
2003	Liu et al., Transgenic Res 12:71-82	Waxy gene	antisense	756 bp	
2004	Popescu and Tumer, Plant J 39:29-44	ribosomal protein L3	inverted repeat	326 bp	
2004	Steppuhn et al., PLoS Biol 2:E217	putrescine N-methyl transferase	inverted repeat	0.9 kb	
			antisense	0.9 kb	
2005	Nunes-Nesi et al., Plant Physiol 137:611–622	mitochondrial malate dehydrogenase	antisense	996 bp	
2006	Gavilano et al., J Agric Food Chem 54:9071-9078	Nicotine N-demethylase	inverted repeat	99 bp	same construct as in Siminszky et al. 2005
			inverted repeat	298bp	
2006	Cao et al., Plant Cell Rep 24:715-23	cytochrome P450 CYP86MF	antisense		
2006	Lou and Baldwin, Plant Physiol 140:126-36	germin-like protein	antisense	250 bp	
			VIGS	250 bp	
2006	Diretto et al., BMC Plant Biol 6:13	lycopene epsilon cyclase	antisense	0.75 Kb	
2006	Yang et al., Plant Mol Biol 62385-95	chloroplast HSP100/ClpB	antisense		
2007	Gibon et al., Plant J 50:093-106	fumarate hydratase	antisense		
2007	Potocky et al., New Phytol	pollen specific NADPH oxidase	antisense oligonucleotides	18 b	direct introduction of synthetic oligonucleotides